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Development and Validation of Reverse Phase High-Performance Liquid Chromatographic Method for Analysis of Flupirtine Maleate in Tablet Formulation

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Abstract: *A simple, rapid, and reliable Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the quantitative determination of Flupirtine Maleate (FLU) in tablet formulations. The chromatographic separation was achieved on a C18 column (Thermo Hypersil gold, 4.6 x 250 mm, 5 µm) using an isocratic mobile phase consisting of 10 mM Phosphate buffer (pH 3.5) and Acetonitrile (60:40 % v/v) at a flow rate of 1.0 ml/min. The eluent was monitored at 250 nm. The method was validated according to ICH guidelines for system suitability, linearity, range, accuracy, precision, ruggedness, robustness, and specificity. The retention time of Flupirtine Maleate was approximately 4.6 minutes. The method exhibited excellent linearity over the concentration range of 80-180 µg/ml (R2=0.9997). Accuracy, determined by the standard addition method, showed mean recoveries ranging from 99.90% to 99.98%. The method demonstrated good precision with a system precision of 0.39% RSD and a method precision of 0.03% RSD. Ruggedness and robustness studies confirmed the reliability of the method under varied conditions. The method was specific, with no interference from placebo components. The developed and validated RP-HPLC method is suitable for routine quality control analysis of Flupirtine Maleate in tablet formulations.*

Keywords: Flupirtine Maleate, HPLC, Method Development, Validation, Pharmaceutical Analysis, Quality Control

I. INTRODUCTION

Flupirtine Maleate, characterized by its specific chemical structure and IUPAC nomenclature, is a pharmaceutical compound widely recognized for its analgesic and muscle relaxant properties, making it a valuable therapeutic agent in various clinical settings. Its precise quantification in pharmaceutical formulations, such as tablets, is of paramount importance to guarantee the quality, safety, and efficacy of the final product administered to patients. This necessitates the development and validation of robust and reliable analytical methodologies capable of accurately determining the amount of Flupirtine Maleate present in these formulations.

In the pharmaceutical industry, analytical methods play a pivotal role throughout the lifecycle of a drug product, from its initial development to routine quality control. These methods are essential for ensuring the consistent quality of raw materials, intermediate products, and finished pharmaceutical products. High-Performance Liquid Chromatography (HPLC) stands out as a versatile and powerful analytical technique frequently employed in pharmaceutical analysis due to its inherent sensitivity, selectivity, and adaptability to a wide range of analytes. The development and validation of an RP-HPLC method for Flupirtine Maleate is crucial for ensuring the availability of a reliable tool for quality assessment.

While existing analytical methods for Flupirtine Maleate might be documented, a critical evaluation of these methods is essential to identify any potential limitations or areas for improvement. Factors such as sensitivity, specificity in the presence of common excipients, analysis time, and method robustness are crucial considerations for routine quality control applications. Therefore, this study focuses on the development and comprehensive validation of a Reverse

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Phase High-Performance Liquid Chromatography (RP-HPLC) method specifically tailored for the quantitative determination of Flupirtine Maleate in tablet formulations. The primary objective is to establish a method that is simple, accurate, precise, specific, robust, and cost-effective, adhering to internationally recognized guidelines for method validation.

II. MATERIALS AND METHODS

2.1.1. Materials and Instruments:

2.1.1.1. Materials:

The Flupirtine Maleate (FLU) pure drug was obtained from Arrow Chem Mumbai with a reported purity of 99.6 % w/w (Table No. 1).

Drug	Supplied by	Quantity	Purity (Assay)
Flupirtine	Arrow Chem Mumbai.	10 g	99.6 % w/w

Table No. 1: Details of API

The marketed tablet preparation ERIRIP 100, containing 100 mg of Flupirtine Maleate per tablet, manufactured by Eridanus Healthcare, was procured from the local market (Table No. 2).

Brand Name	Mfd by	Content	Quantity
ERIRIP 100	Eridanus Healthcare	100 mg	10 tablets

Table No. 2: Details of marketed Preparation

All reagents and chemicals used were of AR grade and HPLC grade: Methanol (HPLC grade), Acetonitrile (HPLC grade), Disodium hydrogenphosphate (AR grade), Distilled Water (HPLC grade), Triethylamine (HPLC grade), and Ortho Phosphoric Acid (HPLC grade).

2.1.1.2. Instruments: The instruments used in the study are listed in Table No. 3,

Sr.No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

Table No. 3: Instruments Used

2.1.2. Study of Functional Group by Using Infrared Spectroscopy:

3 mg of Flupirtine Maleate API was thoroughly mixed with 300 mg of dried KBr and triturated. The mixture was placed in a die, and the IR spectrum was recorded using the Diffused Attenuated Reflectance mode (Fig. No.1).

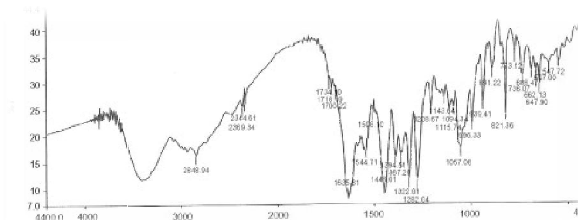

Fig. No.1: IR Spectra of Flupirtine

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Conclusion: The IR spectrum of the test drug matched the reference IR spectrum of Flupirtine (Fig. No. 2).

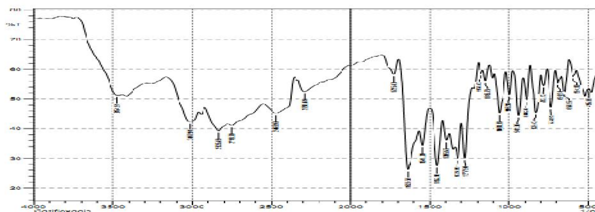

Fig. No. 2: Reference IR Spectra of Flupirtine

2.1.3. Determination of Wavelength Maxima:

A Flupirtine standard stock solution (500 µg/ml) was prepared by dissolving 5 mg of FLU in 10 ml of HPLC grade ACN. A working solution of 5 µg/ml was obtained by further dilution with ACN.

The solution was scanned using a UV-Visible spectrophotometer in the range of 400-200 nm against an ACN blank. The wavelength maxima (λmax) for FLU was found to be 250 nm (Fig. No. 3).

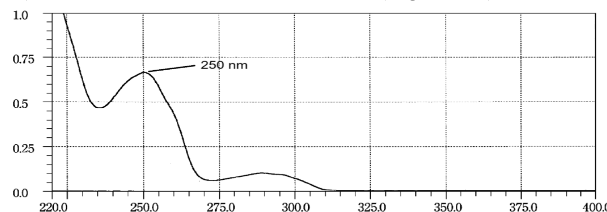

Fig. No. 3: Wavelength Maxima for Flupirtine

2.2. Development of HPLC Method for Estimation of Flupirtine:

2.2.1. Method Development Strategy:

2.2.1.1. Selection of Common Solvent (Diluent): Acetonitrile (HPLC grade) was selected as the common solvent and diluent based on the solubility of Flupirtine Maleate.

2.2.1.2. Preparation of Standard Stock Solution: 1.5 mg of FLU was accurately weighed and dissolved in 100 ml of ACN to prepare the standard stock solution.

Preparation of Diluent: Acetonitrile (HPLC grade) was used as the diluent for preparing stock and working solutions.

Procedure: The mobile phase was allowed to equilibrate with the stationary phase until a stable baseline was achieved. Standard solutions of FLU were injected under different solvent combinations to obtain a stable peak with good characteristics. All solutions were filtered through a 0.15 µm membrane filter. Various mobile phase compositions were evaluated to achieve acceptable separation and peak symmetry under selected chromatographic conditions. The optimized chromatographic conditions were kept constant throughout the method.

2.2.2. Chromatographic Parameters:

Column: C18 (Thermo Hypersil gold) /4.6 x 250 mm, 5µm particle size

Flow Rate: 1.0 ml/min

Wavelength: 250 nm

Injection Volume: 20 µl

Column Oven Temperature: Ambient (25°C)

Run Time: 10 minutes

Mobile Phase: 10mM Phosphate buffer (pH 3.5) and ACN (60:40 % v/v)

Preparation of 10mM phosphate buffer: 1.36 g of potassium dihydrogen phosphate was accurately weighed and dissolved in 1000 ml of HPLC grade water. The pH was adjusted to 3.5 using 1 M Ortho Phosphoric Acid solution.

Fig. No. 4: Separation of FLU in selected mobile phase showing retention time at 4.602 min.

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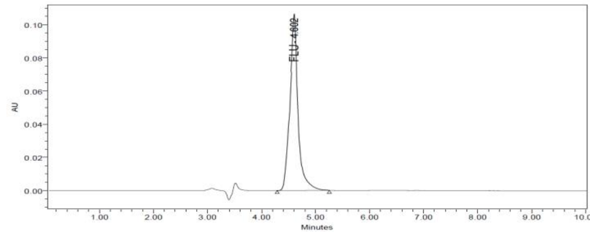

Fig. No. 4: Separation of FLU in selected mobile phase showing retention time at 4.602 min.

2.3. Method Validation:

2.3.1. System Suitability Studies:

System suitability was evaluated by injecting five replicates of the standard working solution of FLU under the optimized chromatographic conditions after equilibration of the mobile phase. System suitability parameters were recorded (Table No.4).

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	FLU	FLU	FLU	FLU
1	440280	4.609	1.10	7526
2	448970	4.608	1.20	7688
3	445895	4.720	1.10	7545
4	442670	4.620	1.15	7500
5	447870	4.650	1.10	7550
Mean	445137	4.641	1.13	7561
S.D	3620	0.04	0.04	73.22
%R.S.D.	0.81	1.01	2.45	0.96

Table No.4: Result of System suitability test

Fig. No.5: Separation of FLU in selected mobile phase showing retention time at 4.546 min.

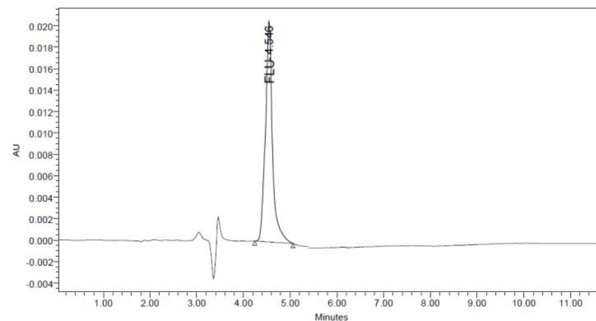

Fig. No 5: Separation of FLU in selected mobile phase showing retention time at 4.546 min.

2.3.2. Application of Proposed Method for Estimation of FLU in Tablet Formulation:

Preparation of Standard Solutions:

Standard Stock Solution: 100 mg of FLU was dissolved in ACN and diluted to 100 ml (1000 µg/ml).

Standard Working Solution: 1 ml of the stock solution was diluted to 10 ml with ACN (100 µg/ml).

Sample Solution Preparation: Ten ERIRIP® tablets (100 mg each) were weighed, and their average weight was calculated. The entire content of one tablet was transferred to a 100 ml volumetric flask, and the volume was made up

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to the mark with ACN (1000 µg/ml). The solution was centrifuged at 5000 rpm for 10 min and filtered through a 0.45 µm membrane filter. 1 ml of the filtrate was diluted to 10 ml with ACN (100 µg/ml).

Procedure: Equal volumes (20 µl) of standard and sample solutions were injected separately after equilibration. The peak areas were measured, and the amount of FLU in the tablet was calculated using the formula: Assay(mg/tablet)=As×Wt×DtAt×Ws×Ds×P×AverageTabletWeight Where:

At = Area count for sample solution
As = Area count for standard solution
Ds = Dilution factor for standard
Dt = Dilution factor for sample
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)
P = Potency of drug (99.6%)

Fig. No. 5: Chromatogram of marketed formulation showing retention time 4.493 min.

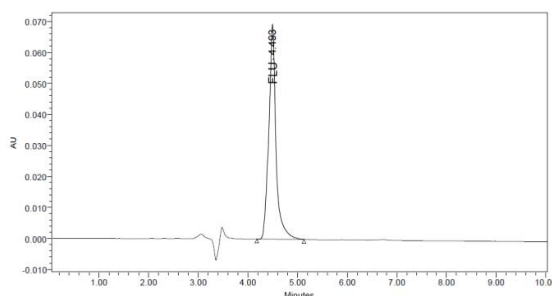

Fig. No.5: Chromatogram of marketed formulation showing retention time 4.493 min.

Table No.5: Results and statistical data for estimation of FLU in marketed formulation

Sr.No.		
	Assay (mg)	% Purity
1	99.98	99.98
2	99.99	99.99
3	99.93	99.93
4	99.95	99.95
5	99.98	99.98
Average	99.96	99.96
SD	0.025	0.025
% RSD	0.025	0.025

Table No. 5: Results and statistical data for estimation of FLU in marketed formulation

III. RESULTS AND DISCUSSION

The High-Performance Liquid Chromatography (HPLC) technique is highly valuable for determining active pharmaceutical ingredients in formulations. This study focused on developing an HPLC method for the analysis of Flupirtine Maleate (FLU) in its tablet formulation.

Flupirtine Maleate is a relatively new analgesic drug used for acute, moderate-to-severe pain. While some methods for its estimation exist, this investigation aimed to develop a simple and efficient HPLC method for routine analysis. Pure standards of FLU were obtained from Arrow Chem Mumbai, with a reported purity of 99.6% w/w (Table No. 6).

Drug	Supplied by	Quantity	Purity (Assay)
Flupirtine	Arrow Chem Mumbai.	10 g	99.6 % w/w

Table No. 6: Details of API

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This reported purity was used as a reference for comparison in this study.

3.1. RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC is a powerful analytical tool due to its ease of use, specificity, sensitivity, and applicability to complex sample matrices. It is widely used for quantitative determination of drugs in formulations and for studying drug metabolites in biological fluids. The multi-component handling capability of HPLC makes it suitable for this investigation aimed at estimating FLU in tablet formulations. Careful optimization of various analytical parameters is crucial during method development. The following aspects were considered for establishing the RP-HPLC method:

3.1.1. HPLC Column Selected:

A Waters 600 HPLC system equipped with a C18 (Thermo Hypersil gold) /4.6 x 250mm, 5µ particle size column and a PDA detector was used for the study. Standard and sample solutions of FLU were prepared in the diluent (Acetonitrile). Different solvent systems with varying polarities and proportions were evaluated as mobile phases during method development.

3.1.2. Mobile Phase Selected:

The optimized mobile phase consisted of a mixture of 10mM Phosphate buffer (pH 3.5) and Acetonitrile (ACN) in a ratio of 60:40 % v/v. An isocratic elution program with a total run time of 10 minutes was employed. The detection wavelength was set at 250 nm, which corresponds to the \\lambda_\{max\} of FLU determined by UV scanning of the standard solution. This chromatographic system provided good resolution, an optimal retention time (approximately 4.6 minutes as seen in system suitability), and an acceptable tailing factor (< 2). The chromatographic conditions established through trial and error were kept constant throughout the method (Table No. 7).

Column	C_{18} (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	250 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	10mM Phosphate buffer (pH 3.5) and ACN (60:40 % v/v)

Table No.7: Chromatographic Parameters

Mobile phase preparation:1.36 g of potassium dihydrogen phosphate was accurately weighed and dissolved in 1000 ml of HPLC grade water. The pH was adjusted to 3.5 using 1 M Ortho Phosphoric Acid solution.

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	FLU	FLU	FLU	FLU
1	440280	4.609	1.10	7526
2	448970	4.608	1.20	7688
3	445895	4.720	1.10	7545
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5	447870	4.650	1.10	7550
Mean	445137	4.641	1.13	7561
S.D	3620	0.04	0.04	73.22
%R.S.D.	0.81	1.01	2.45	0.96

Table No. 8: Summary of system suitability of Test results

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Preparation of diluent:Acetonitrile (HPLC grade) was selected as the common solvent for preparing stock solutions and for spectral characterization. Further dilutions from stock solutions were made in the mobile phase.
The system suitability results (Table No.8) demonstrate that the chromatographic system is performing adequately for the intended analysis.

After establishing the chromatographic conditions, standard and marketed tablet preparation solutions were prepared and analyzed as described in the experimental work. The method yielded accurate and reliable results and was successfully applied for the estimation of FLU in the marketed tablet formulation. The amount of drug in the tablet was calculated using the provided formula.

3.1.3. VALIDATION

The developed HPLC method was validated according to USP guidelines for the following parameters:

3.1.3.1 Precision:

System Precisio The system precision was evaluated by injecting three replicates of the standard solution. All system suitability parameters were found to be within the acceptable limits (Table No.9).

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.45	NMT 2.0
2	Theoretical plates	7560	NLT 2000
3	Tailing factor	1.36	NMT 2.0

Table No. 9: Data showing system Precision

Method Precision:Replicate analysis of the tablet formulation using the proposed method yielded consistent results, indicating good repeatability. The Relative Standard Deviation (RSD) was found to be less than 2% (Table No. 10).

Sr.no.	FLU	
	Assay (mg)	Assay (mg)
1	99.98	99.98
2	99.99	99.99
3	99.97	99.97
Average	99.98	99.98
SD	0.02	0.02
% RSD	0.03	0.03

Table No.10: Method Precision Studies Set – I

3.1.3.2 Linearity & Range:

The linearity of the analytical procedure was established over a concentration range of 80% to 150% of the target concentration, with five concentration levels. A graph of concentration versus mean peak area showed excellent correlation, with an R^2 value of 0.999 for FLU. This indicates a direct proportionality between concentration and peak area within the tested range, confirming the linearity of the method.

3.1.3.3 Accuracy:

The accuracy of the proposed method was determined by recovery studies using the standard addition method. The recovery results, ranging from 99.90% to 99.98% (Table No.11), are well within the acceptable limits (98.0% to 102.0%), indicating that the method is accurate.

	FLU		
	Levels		
	80%	80%	80%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.99	99.99	119.95
	79.95	99.98	119.98
	79.98	99.97	119.98
% Recovery	99.98	99.98	99.95
	99.93	99.98	99.98
	99.97	99.97	99.98
Mean % recovery	99.90	99.98	99.93
% RSD	0.15	0.28	0.01

Table No. 11: Result of Accuracy Studies

3.1.3.4 Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.

Effect of variation in flow rate of mobile phase by ± 10%

Organic phase composition (± 10%)

Change in Wavelength by ± 2 units

3.1.3.5 Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of FLU. Thus, no interference was found at the Retention time of FLU.

IV. SUMMARY AND CONCLUSION:

4.1. Summary:

Flupirtine Maleate (FLU) is an analgesic drug used for the treatment and management of pain, and tablet formulations containing FLU have been introduced in the market. A review of the literature revealed a limited number of analytical methods available for the estimation of FLU.

The present study was conducted with the objective of developing a suitable, sensitive, and simple Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the estimation of FLU in tablet formulations.

In the developed RP-HPLC method, the analyte was separated using a mobile phase composed of 10mM Phosphate buffer (pH 3.5) and Acetonitrile (ACN) in a ratio of 60:40 %v/v. An isocratic elution program with a total run time of 10 minutes was employed using an HPLC auto-sampler system containing a PDA detector with EMPOWER Software and a C18 (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size column. The detection wavelength was set at 250 nm. The developed method provided good resolution and a suitable retention time for FLU.

The results obtained from the method were validated in terms of accuracy, precision, ruggedness, linearity, and range. The method was found to be sensitive, reliable, reproducible, rapid, and economical.

4.2. Conclusion:

Based on the results of this study, it can be concluded that the developed RP-HPLC technique was successfully applied for the estimation of Flupirtine Maleate in the tablet formulation.

The method demonstrated good reproducibility, accuracy, precision, specificity, and sensitivity. The analysis of the tablet formulation of FLU was effectively performed using the developed and validated RP-HPLC method.

The RP-HPLC method is also simple, accurate, precise, reproducible, and economical, making it suitable for routine quality control analysis of FLU in tablet formulations. No interference from common tablet additives or the formulation matrix was observed. Further studies on other pharmaceutical formulations containing FLU could provide more insights. Additionally, the suitability of this method for the analysis of biological samples warrants further investigation.

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